Bulletin du Muséum national d'Histoire naturelle, Paris, 4e série, **18**, 1996
Section A, n°s 3-4 : 547-555

Spermatophore morphology and spermatozoal ultrastructure of the recently described hermit crab, *Strigopagurus boreonotus* Forest, 1995 (Decapoda, Anomura, Diogenidae)

by Christopher C. TUDGE

Abstract. — The spermatophore morphology and spermatozoal ultrastructure of the diogenid hermit crab, *Strigopagurus boreonotus*, is described and compared with that of previously investigated diogenid genera. The spermatophores show similarities with those described for the genera *Calcinus* and *Dardanus*. The spermatozoa have an overall morphology which is reminiscent of representatives in the genus *Clibanarius*, above all, the genus *Calcinus*, *while still retaining a particular suite of spermatozoal characters so far unique to Strigopagurus*.

Key-words. — Spermatozoa, spermatophores, ultrastructure, *Strigopagurus*, Diogenidae.

Morphologie du spermatophore et ultrastructure du spermatozoïde du bernard-l'hermite récemment décrit, *Strigopagurus boreonotus* Forest, 1995 (Decapoda, Anomura, Diogenidae)

Résumé. — La morphologie du spermatophore et l'ultrastructure du spermatozoïde du bernard-l'hermite Diogenidae, *Strigopagurus boreonotus*, sont décrites et comparées avec celles des autres genres de Diogenidae précédemment étudiés. Le spermatophore montre des similarités avec ceux décrits dans les genres *Calcinus* et *Dardanus*. Le spermatozoïde a une morphologie générale qui rappelle les espèces du genre *Clibanarius* et surtout du genre *Calcinus*, tout en montrant un ensemble original de caractères spermatologiques trouvés jusqu'ici seulement chez *Strigopagurus*.

Mots-clés. — Spermatozoïde, spermatophores, ultrastructure, *Strigopagurus*, Diogenidae.

C. C. TUDGE, *Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington D C 20560, USA, current address: Crustacean Laboratory, Museum of Victoria, 71 Victoria Crescent, Abbotsford, Vic. 3067, Australia.*

INTRODUCTION

The anomuran family Diogenidae is a morphologically diverse taxon currently encompassing eighteen genera. Although comprised of a heterogeneous assemblage of genera the family is considered to be an ancient monophyletic group (FOREST 1995). Genera are often characterised by a combination of morphological characters but each character can vary among the other members of the family. Single generic apomorphies are rare. This lack of good generic apomorphies has made elucidating the phylogenetic relationships between the different genera in the Diogenidae very difficult (FOREST 1984, 1995) and has led FOREST to state "*... qu'il est difficile d'éclaircir la phylogénie des Diogenidae et de préciser les liens de parenté entre des genres si disparates.*" (FOREST 1995; 28).

This diversity of adult morphological form in the Diogenidae is reflected in the equally diverse morphology of reproductive components such as spermatophores (MOUCHET 1930, 1931; HAMON 1939; MATTHEWS 1953, 1956, 1957; TUZET & MANIER 1961; UMA & SUBRAMONIAM 1984; TUDGE 1991, 1995a) and spermatozoa (KOLTZOFF 1906; NATH 1942; DHILLON 1964, 1968; JAMIESON 1991; TUDGE 1992; TUDGE & JUSTINE 1994; TUDGE 1995a, b). With the increasing use of transmission electron microscopy to study the ultrastructural characters of spermatophores and spermatozoa within the Diogenidae (and the Anomura in general) some doubt has been cast on the monophyly of the family (TUDGE 1991, 1992, 1995a, b).

The present study describes and illustrates the spermatophore and spermatozoal ultrastructure from the holotype specimen of the diogenid *Strigopagurus boreonotus* and compares it with the ultrastructure of previously studied species in other diogenid genera.

MATERIAL AND METHODS

The specimen of *Strigopagurus boreonotus* Forest, 1995 was collected by Dr B. Richer de Forges during the *Bathus 2* cruise off the west coast of New Caledonia (22°46'S – 167°14'E), south-west Pacific in May 1993. It was collected at station number 718 at a depth of 430-436 m. This specimen is now the designated holotype (MNHN-Pg 5181).

The male reproductive material (testes and the ducts of the vasa deferentia) was removed from the single male specimen and fixed in cold glutaraldehyde for a minimum of two hours at 4 °C then posted to Brisbane (Queensland, Australia) at ambient temperature where the remainder of the fixation and embedding process was carried out.

For light microscopy, glutaraldehyde-fixed spermatophores were viewed under an Olympus BH2 Nomarski interference contrast microscope. Micrographs were taken on an attached Olympus OM-2 camera.

TRANSMISSION ELECTRON MICROSCOPY

The gonad tissue of *Strigopagurus boreonotus* was processed in the Zoology Department, The University of Queensland, by the standard fixation procedure (outlined below) for transmission electron microscopy. This was carried out in a Lynx-el. Microscopy Tissue Processor, after the initial glutaraldehyde fixation and first phosphate buffer wash.

Portions of the testis (approximately 1 mm^3) were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2), with 1-3% sucrose added, for a minimum of one hour at 4 °C. They were washed in phosphate buffer (3 washes in 15 min), postfixed in phosphate buffered 1% osmium tetroxide for 80 min; similarly washed in buffer and dehydrated through ascending concentrations of ethanol (40-100%). After being infiltrated and embedded in Spurr's epoxy resin, thin sections (50-80 nm thick) were cut on a LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilized colloidin-coated 200 µm mesh copper grids and stained (according to DADDOW 1986) in Reynold's lead citrate for 30 s, rinsed in distilled water, then 6% aqueous uranyl acetate for 1 min, Reynold's lead citrate again for 30 s and a final rinse in distilled water. Micrographs were taken on an Hitachi H-300 transmission electron microscope at 80 kV.

RESULTS

Spermatophore morphology

The pedunculate spermatophores of *Strigopagurus boreonotus* are composed of an ovoid to spherical, sperm-filled ampulla (220 µm long × 170 µm wide) attached to a long, relatively thick stalk (Fig. 1). The spermatophores are large, being approximately 850 µm in length.

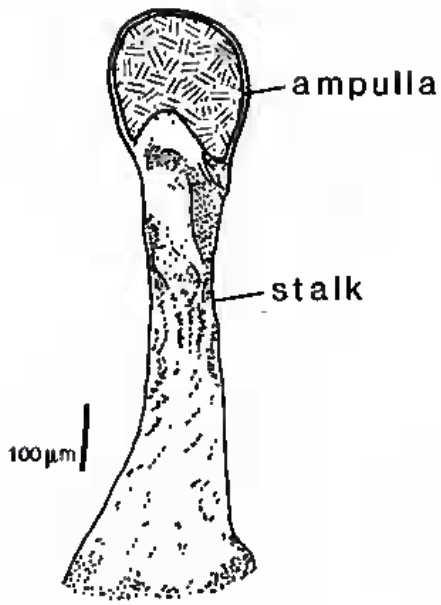

Fig. 1. — *Strigopagurus boreonotus* (Diogenidae). Semidiagrammatic representation of a spermatophore. Traced from a light micrograph. Scale bar as shown.

Spermatozoal morphology

The spermatozoa are composed of a spherical acrosome vesicle capped by a convexly domed operculum and penetrated posteriorly by an extensive perforatorial chamber. The acrosome vesicle has a length of 5.3 µm and is 4.5 µm wide. Posterior to the acrosome vesicle is the cytoplasmic region with three microtubular arms and, more posteriad, the nucleus. The entire sperm cell is approximately 10 µm in length (refer to Figs 2, 3 throughout).

Acrosome

The apical or anterior pole of the acrosome vesicle of *Strigopagurus boreonotus* is covered by a high domed, electron-dense operculum (Fig. 3A, D). Subjacent to, and filling the inside of, the domed operculum is a coarsely granular, homogeneous subopercular zone. The subopercular zone extends posteriorly to meet the perforatorial chamber and laterally to abut on the inner and outer acrosome zones (Fig. 3A). Along this boundary a thin, electron-lucent area containing small electron-dense granules occurs. Thinly enveloping the anterior end of the perforatorial chamber is the inner acrosome zone. This homogeneous, finely granular zone forms an

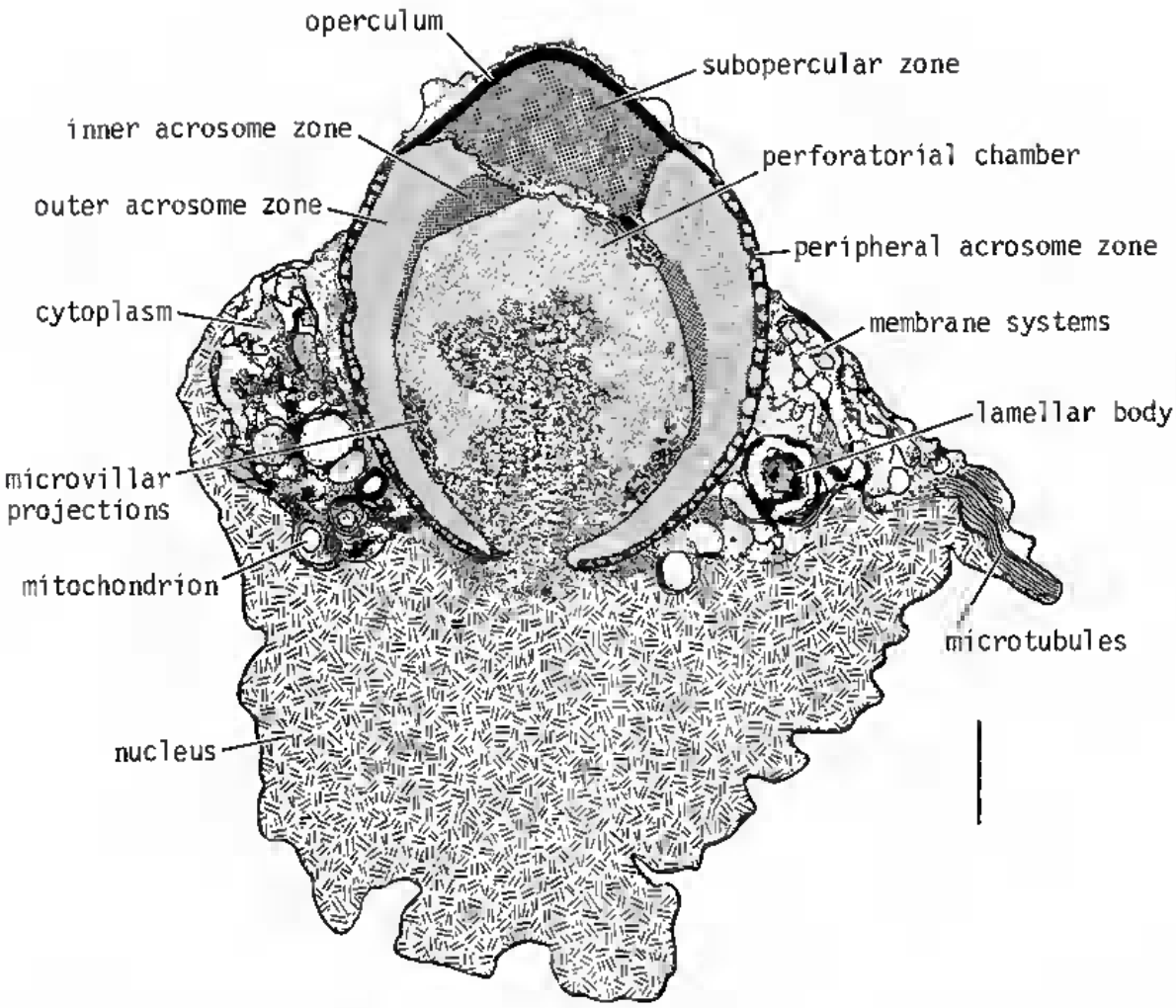

FIG. 2. — *Strigopagurus boreonotus* (Diogenidae). Semidiagrammatic longitudinal section of a spermatozoon, based on a tracing of a micrograph. Scale bar = 1 µm.

electron-dense ring which extends no further posteriad than the widest point of the perforatorial chamber (Fig. 3A, E). External to the inner acrosome zone, and comprising the bulk of the acrosome vesicle, is the outer acrosome zone. This zone is similar in form to the inner acrosome zone but less electron-dense. The outer acrosome zone is prevented from reaching the exterior acrosome membrane by the intervention of a thin, moderately electron-dense zone which lies beneath the acrosome membrane, the peripheral acrosome zone (Fig. 3A, E, F). This peripheral zone has a slightly irregular appearance (pale lacunae in a darker matrix) and extends from the operculum to the basal opening of the perforatorial chamber.

The invaginated perforatorial chamber is spherical, about 3 µm at its widest point and occupies a large portion of the centre of the sperm cell (Fig. 3A, E, F). It has a constricted basal opening and the anteriormost (apical) region can vary in form, giving the appearance of

some asymmetry. Posteriorly, the walls of the perforatorial chamber produce short, microvillar projections which extend laterally into the chamber (Fig. 3A, E). The contents of the perforatorial chamber is divisible into two areas of differing form and the more posterior region appears continuous with the cytoplasm in the region of the constricted opening. The posteriormost portion is coarsely granular, heterogeneous and can appear almost reticulate. This region changes anteriorly to give a more homogeneous, finely granular zone. The boundary between the two areas is approximately at the midpoint of the perforatorial chamber (Fig. 3A).

Cytoplasmic region

The cytoplasm forms a thick collar around the posterior part of the acrosome vesicle, although a thin layer also occurs beneath the vesicle. Abundant membranes and membrane systems associated with numerous cristate mitochondria are a conspicuous part of the cytoplasm (Fig. 3A, C, F). A single, concentrically arranged, membraneous whorl or lamellar scroll is apparent in many of the spermatozoa (Fig. 3A, C). Bundles of microtubules representing the bases of the three microtubular arms pass through the cytoplasm and a pair of centrioles is seen in the cytoplasm directly below the constricted opening of the perforatorial chamber (Fig. 3B). A disrupted nuclear membrane forms a discontinuous partition between the two regions.

Nuclear material

The nucleus is amorphous, but maintains an approximately globular shape with a crenulated external surface, and is surrounded by a thickened nucleo-plasma membrane. The contents of the nucleus are finely granular, relatively homogeneous and electron-pale (Fig. 3A).

DISCUSSION

The spermatophore morphology described for *Strigopagurus boreonotus* is consistent with the pedunculate spermatophore type common in, and perhaps diagnostic of, the Anomura (Tudge 1995a). This pedunculate spermatophore morphology is a tripartite arrangement with a sperm-filled ampulla connected to a pedestal or basal plate via a stalk.

Within the Diogenidae the spermatophore morphology has been recorded from twenty-two species in eight genera (see list of references in the Introduction) and from these representatives the morphology of the spermatophore of *Strigopagurus boreonotus* (Fig. 1) appears most similar to the genera *Calcinus* and *Dardanus*, and especially that described for *Dardanus megistos* (Tudge 1991, 1995a); except that the spermatophore of *S. boreonotus* is nearly six times larger. The importance of size of spermatophores as a reliable distinguishing character in the Anomura is undermined by the fact that spermatophore size is proportional to the diameter of the vas deferens of the reproductive tract which is in turn proportional to the size of the individual specimen. Despite this fact, the size of the spermatophore in *S. boreonotus* (850 µm) more closely approximates the range of sizes recorded for representatives of the genus *Dardanus* (160-3500 µm) than those recorded for the genus *Calcinus* (150-180 µm) (Tudge 1991, 1995a). The

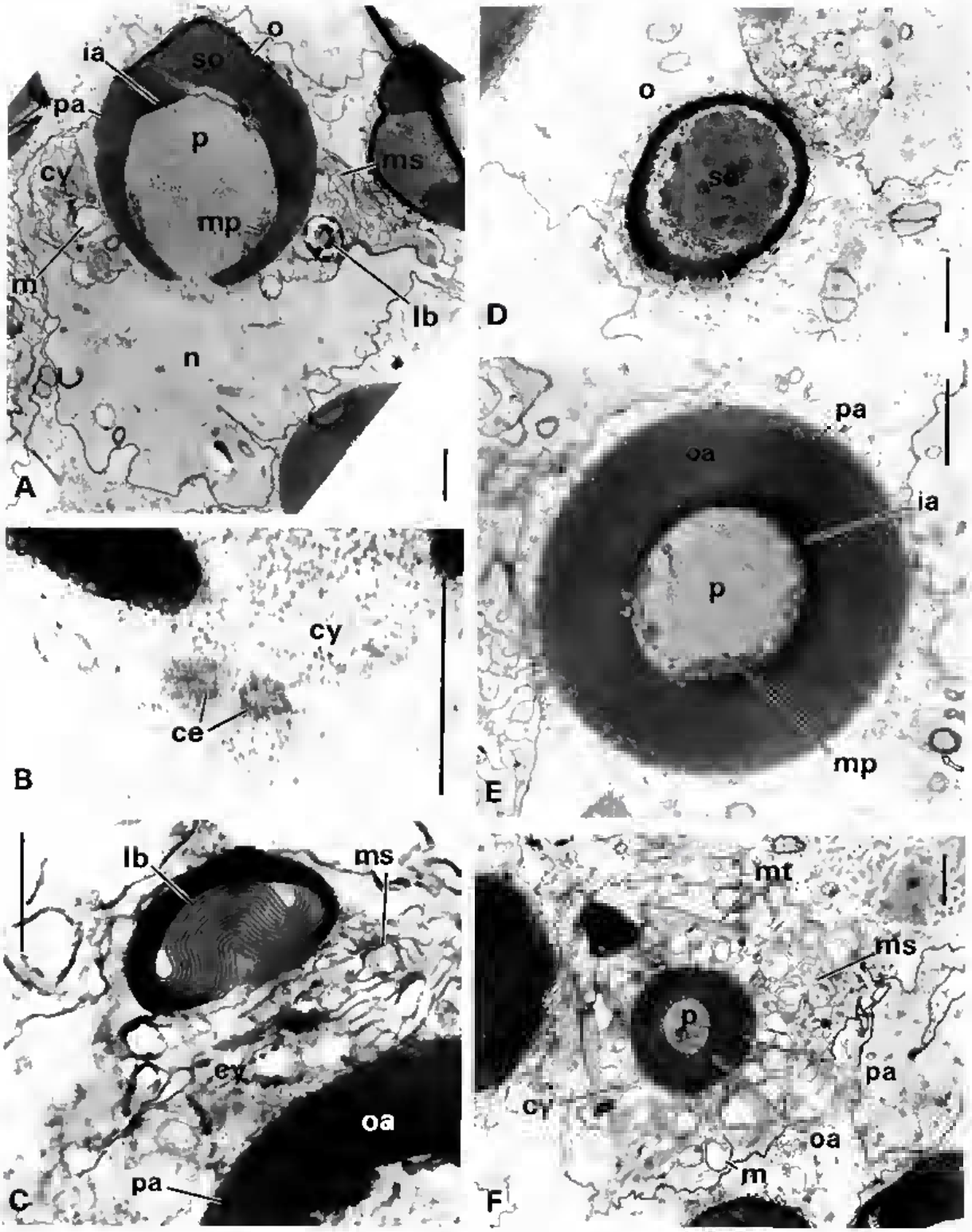

FIG. 3. — *Strigopagurus boreonotus* (Diogenidae). A-F, transmission electron micrographs of spermatozoa. A, Longitudinal Section (LS) of a spermatozoon. B, detail of LS of base of acrosome vesicle and cytoplasm showing two centrioles. C, detail of cytoplasm showing conspicuous lamellar body. D-F, transverse sections through acrosome vesicle at the level of the operculum (D), the microvillar projections (E) and the base of the acrosome vesicle and cytoplasm (F). Abbreviations: ce, centriole; cy, cytoplasm; ia, inner acrosome zone; lb, lamellar body; m, mitochondrion; mp, microvillar projections; ms, membrane system; mt, microtubules; n, nucleus; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; so, subopercular zone. Scale bars = 1 µm.

spermatophore morphology of *S. boreonotus* is unlike any morphology recorded for other paguroids (outside the Diogenidae) or even other anomurans (TUDGE 1991, 1995a).

The spermatozoa of *Strigopagurus boreonotus* conform to a general paguroid sperm type characterised by a concentrically zoned acrosome vesicle, apically capped by an electron-dense operculum; the acrosome vesicle shape may vary from spherical, through ovoid, to more elongate and cylindrical; the acrosome vesicle is penetrated from its posterior end by a perforatorial chamber, which may terminate pre-equatorially or extend to a subterminal position immediately beneath the operculum; the acrosome vesicle is embedded in the cytoplasm and/or nucleus and, most importantly, there are three microtubular arms (of cytoplasmic origin) which emerge from the cytoplasm below the acrosome vesicle (POCHON-MASSON 1968a, b; CHEVAILLIER 1970; HINSCH 1980; JAMIESON 1991; TUDGE & JAMIESON, 1991; TUDGE 1992, 1995a, b).

In size and shape the spermatozoa of *Strigopagurus boreonotus* are similar to investigated representatives in the genera *Calcinus* and *Clibanarius*; although many ultrastructural differences are apparent (JAMIESON 1991; TUDGE 1992, 1995a, b). The position of the thin inner acrosome zone on the anterior region of the perforatorial chamber in *S. boreonotus* (Figs 2, 3A, E) is similar in *Calcinus minutus* and the bulbous, almost spherical perforatorial chamber with small microvillar projections (Figs 2, 3A, E, F) is approximated by all three investigated species in the genus *Calcinus* (TUDGE 1995a, b). The tendency for the anterior wall of the perforatorial chamber in *S. boreonotus* to be irregular (Figs 2, 3A) may indicate links to the investigated *Calcinus* species in which this region of the perforatorial chamber divaricates to form two or more distinct fingers or lobes (TUDGE 1995a, b). This latter character's presence is an autapomorphy for the genus *Calcinus*. There is no dense perforatorial ring, an autapomorphy of the investigated *Clibanarius* species, seen in the spermatozoa of *S. boreonotus*. Some (apomorphic?) structures of the acrosome vesicle present in the sperm of *S. boreonotus* which have not been seen in other investigated diogenids are the extreme width and bulbous shape of the perforatorial chamber, the electron-lucent, granular region forming the boundary between the perforatorial chamber and subopercular zone and the loculated appearance of the peripheral acrosome zone (Figs 2, 3A, E, F).

The spermatozoa of *Strigopagurus boreonotus* have a distinct ultrastructural morphology which distinguishes this species from the other investigated genera in the Diogenidae. Some overall similarities to investigated members in the genus *Clibanarius* and also representatives in the genus *Calcinus* are apparent. A preliminary phylogenetic analysis based on spermatozoal and spermatophore characters (analysis in progress, results available upon request) consistently placed *Strigopagurus boreonotus* in the same clade with, and between, the genera *Calcinus* and *Clibanarius* (TUDGE 1995a). At present, only six of the eighteen genera in the Diogenidae have been investigated for spermatophore and spermatozoal morphology. With future research into the remaining genera, spermatophore and spermatozoal ultrastructural morphology may further assist in the elucidation of inter-generic relationships within the family Diogenidae.

Acknowledgements

I wish to thank Dr Bertrand RICHER DE FORGES (ORSTOM, Nouméa) for the collection and initial fixation of the specimen of *Strigopagurus boreonotus*. Mrs Lina DADDOW (Zoology Department, The University of Queensland) is heartily thanked for expert assistance with all aspects of electron microscopy.

I am also grateful to Prof. Jacques FOREST (MNHN, Paris) for identification of the specimen. The use of facilities at the Natural History Museum, Washington D.C., is gratefully acknowledged.

REFERENCES

CHEVAILLIER P., 1970. — *Recherche sur la structure et les constituants chimiques des cellules germinales mâles des Crustacés Décapodes*. Doctor of Science Thesis, University of Rennes, Rennes, France : 1-323.

DADDOW L. Y. M., 1986. — An abbreviated method of the double lead stain technique. *J. Submicrosc. Cytol.* **18**: 221-224.

DHILLON B., 1964. — Sperm nucleus of *Chibanarius longitarsus*. *Experientia* **20**: 505-506.

— 1968. — Radial processes of decapod sperm. *Microscope* **76**: 365-368.

FOREST J., 1984. — Révision du genre *Aniculus* (Decapoda, Diogenidae). *Crustaceana* (Suppl. **8**) : 1-91.

— 1995. — Crustacea Decapoda Anomura: révision du genre *Trizopagurus* Forest, 1952 (Diogenidae), avec l'établissement de deux genres nouveaux. *In* A. CROSNIER (ed.). Résultats des Campagnes MUSORSTOM, Vol. 13. *Mém. Mus. natl Hist. nat* **163** : 9-149.

HAMON M., 1939. — La constitution chimique des spermatophores de Crustacés supérieurs du groupe des Pagurides. *C. R. Soc Biol.* **130** : 1312-1314.

HINSCH G. W., 1980. — Spermiogenesis in *Coenobita clypeatus*. I. Sperm structure. *Int. J. Invert. Reprod.* **2**: 189-198.

JAMIESON B. G. M., 1991. — Ultrastructure and phylogeny of crustacean spermatozoa. *Mem. Qld. Mus.* **31**: 109-142.

KOLTZOFF N. K., 1906. — Studien über die gestalt der zelle. I. Untersuchungen über die spermien der decapoden, als einleitung in das problem der zellengestalt. *Arch. Mikroskop. Anat.* **67**: 364-572.

MATTHEWS D. C., 1953. — The development of the pedunculate spermatophore of a hermit crab, *Dardanus asper* (De Haan). *Pac. Sci.* **7**: 255-266.

— 1956. — The probable method of fertilization in terrestrial hermit crabs based on a comparative study of spermatophores. *Pac. Sci.* **10**: 303-309.

— 1957. — Further evidences of anomuran non-pedunculate spermatophores. *Pac Sci.* **11**: 380-385.

MOUCHET S., 1930. — Mode de formation des spermatophores chez quelques Pagures. *C. R. Acad. Sci. Paris* **190** : 691-692.

— 1931. — Spermatophores des Crustacés Décapodes Anomoures et Brachyoures et castration parasitaire chez quelques Pagures. *Stat. Océanogr. Salammbô, Ann.* **6** : 1-203.

NATH V., 1942. — The decapod sperm. *Trans. Nat. Inst. Sci. India* **2**: 87-119.

POCHON-MASSON J., 1968a. — L'ultrastructure des spermatozoïdes vésiculaires chez les Crustacés Décapodes avant et au cours de leur dévagination expérimentale. I. Brachyoures et Anomoures. *Ann. Sci. Nat., Zool. Biol.* **10** : 1-100.

— 1968b. — L'ultrastructure des spermatozoïdes vésiculaires chez les Crustacés Décapodes avant et au cours de leur dévagination expérimentale. II. Macroures. Discussion et conclusions. *Ann. Sci. Nat., Zool. Biol.* **10** : 367-454.

TUDGE C. C., 1991. — Spermatophore diversity within and among the hermit crab families, Coenobitidae, Diogenidae and Paguridae (Paguroidea, Anomura, Decapoda). *Biol. Bull.* **181**: 238-247.

— 1992. — Comparative ultrastructure of hermit crab spermatozoa (Decapoda: Anomura: Paguroidea). *J. Crust. Biol.* **12**: 397-409.

— 1995a. — *The Ultrastructure and Phylogeny of Anomuran Crab Spermatozoa*. PhD. Thesis, Zoology Department, The University of Queensland, Australia: 1-346.

— 1995b. — Ultrastructure and Phylogeny of the Spermatozoa of the Infraorders Thalassinidea and Anomura (Decapoda, Crustacea). *In* B. G. M. JAMIESON, J. AUSIO & J.-L. JUSTINE (eds). Advances in Spermatozoal Phylogeny and Taxonomy. *Mém. Mus. natl Hist. nat* **166** : 251-263.

TUDGE C. C. & JAMIESON B. G. M., 1991. — Ultrastructure of the mature spermatozoon of the coconut crab *Birgus latro* (L.) (Coenobitidae, Paguroidea, Decapoda). *Mar. Biol.* **108**: 395-402.

TUDGE C. C. & JUSTINE J.-L., 1994. — The cytoskeletal proteins actin and tubulin in the spermatozoa of four decapod crabs (Crustacea, Decapoda). *Acta Zool.* (Stockholm) **75**: 277-285.

TUZET O. & MANIER J.-F., 1961. — Les constituants cytoplasmiques au cours de la spermatogenèse de six pagures. *Bull. Soc. zool. Fr.* **86** : 305-315.

UMA K. & SUBRAMONIAM T., 1984. — A comparative study of the spermatophore in *Scylla serrata* (Forskal) (Decapoda: Brachyura) and *Clibanarius longitarsus* (De Haan) (Decapoda: Anomura). *J. Mar. Biol. Assoc. India* **26**: 103-108.